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Novel Keratin 14 Mutations in Patients with Severe Recessive Epidermolysis Bullosa Simplex

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TO THE EDITOR

Epidermolysis bullosa simplex (EBS) is the most common subtype, accounting for one-half of all epidermolysis bullosa cases (Pfendner *et al.*, 2005). It is clinically characterized by nonscarring blisters of the skin caused by little or no trauma, and morphologically by intraepidermal blistering. The major subtypes of EBS result from mutations in either the keratin 5 (*KRT5*) or keratin 14 (*KRT14*) gene, whereas mutations in the gene for plectin (*PLEC1*) cause the rare forms, EBS with muscular dystrophy and EBS Ogna. The clinical spectrum of EBS ranges from mild blistering of the hands and feet (EBS Weber–Cockayne) to more generalized blistering (EBS Koebner, EBS Dowling-Meara, and EBS with mottled pigmentation). EBS, similarly to most of the keratin disorders identified in humans, is caused by dominant missense mutations; however, patients with recessive EBS due to keratin mutations have been reported, representing about 5% of all EBS mutations (Porter and Lane, 2003).

In this study, we investigated two unrelated patients with severe neonatal blistering, both offspring of consanguineous, unaffected parents of Turkish (patient 1), respectively German (patient 2) origin. Indirect immunofluorescence (IIF) of the skin cryosections was performed as described (Hammami-Hauasli *et al.*, 1998) with monoclonal antibodies anti-human keratin 5 (clone D5/16 B4, Dako, Hamburg, Germany) and keratin 14 (clone LL002, BioGenex, San Ramon, CA). Genomic DNA was extracted from peripheral blood samples collected from patients and their unaffected parents using the Qiagen Blood DNA Kit (Qiagen, Hilden, Germany). Long-range polymerase chain reaction amplification of the *KRT14* gene was performed as described (Wood *et al.*, 2003) and direct sequencing in both directions was performed, using primers as published by Schuilenga-Hut *et al.* (2003) and an ABI prism 3100 automated sequencer (ABI, Darmstadt, Germany). The study was conducted according to the Declaration of Helsinki Principles**,** and the participants gave their written informed consent. The medical committee of the University of Freiburg approved all described studies.

Patient 1, a 2-year-old boy, showed blistering predominantly on hands and feet since birth (Figure 1a). In the course of the disease, bullae became rarer, occurred mechanically induced also on the head and trunk and healed without scarring. Patient 2, aged 1 year, showed at birth extensive blistering of the hands and feet (Figure 1b) and suffered from congenital pneumonia. Oral

Abbreviations: EBS, epidermolysis bullosa simplex; IIF, indirect Immunofluorescence; KRT, keratin

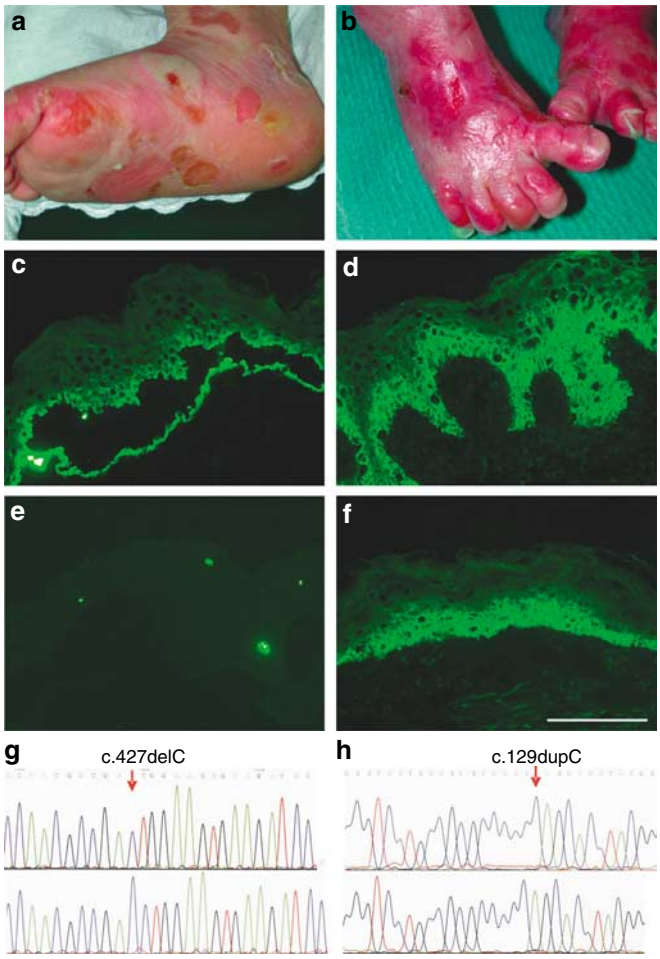

Figure 1. Phenotypes of the patients, indirect immunofluorescence staining of skin sections and mutations in the *KRT14* gene. (**a**) Blisters and erosions on the feet of patient 1 at the age of 2 years. (**b**) Patient 2 at 2 weeks of age showed severe blistering. (**c**) IIF with antibodies against keratin 5 showed intraepidermal splitting in the skin of patient 2. (**d**) IIF of control skin with antibodies against keratin 5. (**e**) IIF staining with antibodies against keratin 14 yielded a negative signal in patient's 1 skin. (**f**) IIF with antibodies against keratin 14 produced a positive signal in the basal keratinocyte layer in control skin. Bar = 100 μm (given in (**f**) but refers to all images). (**g**) Partial sequence of *KRT14* exon 1, showing in the upper panel the mutation c.427delC (red arrow) and in the lower panel the wild-type sequence. (**h**) Partial sequence of *KRT14* exon 1, showing in the upper panel the mutation c.129dupC (red arrow) and in the lower panel the wild-type sequence.

blisters were present in the first days. Later, he developed blisters on arms, legs, and trunk after mechanical trauma. In both cases, IIF staining of skin sections revealed a split level through the basal cell layer (Figure 1c) and antibodies against keratin 14 yielded a negative staining (Figure 1e), suggesting keratin 14 defects in both cases. Mutation detection in *KRT14* revealed in patient 1 a homozygous one base pair deletion in exon 1, designated c.427delC (Figure 1g); this results in a frame shift starting with codon 143 and formation of a premature termination codon, two codons downstream, p.L143fsX2. In patient 2, also in exon 1 of *KRT14*, a homozygous duplication, c.129dupC was disclosed (Figure 1h), leading to a frame shift starting with codon 44 and premature termination codon 38 codons downstream (p.S44fsX38). The parents were found to be heterozygous carriers of the respective mutations.

In both patients, the clinical picture was compatible with the diagnosis of EBS Koebner. Mucosal involvement and other symptoms are difficult to assess because of the very young age and the short time they were available for observation. Absence of keratin 14 from the epidermis was caused by homozygous deletion/duplication mutations leading to premature termination codons. Important information was provided by IIF staining of a skin biopsy: first, intraepidermal blistering was evident and second, the negative signal with keratin 14 antibodies permitted the rapid identification of the candidate gene. Except for the two novel mutations described here, 11 different *KRT14* mutations associated with recessive EBS have been published before (Table 1): four with nonsense mutations, two with missense mutations, one with a splice site mutation, one compound heterozygous for nonsense and missense mutations, one with a deletion/insertion mutation, and two with deletion mutations. The less severely affected case reported by Batta *et al.* (2000) is the recessive case with the shortest expressible *KRT14* sequence to date, predicted to be truncated after only 30 amino acids (Porter and Lane, 2003; patient 6 in Table 1). In our patient 1, the truncation of keratin 14 is predicted to start after 143 amino acids, within the very beginning of the rod domain, and in patient 2 after 44 amino acids, within the head domain of keratin 14. Other authors described severely affected keratin 14 ''knockout'' patients (Table 1). However, no correlation between the position of the premature termination codon and the severity of the phenotype is obvious. Rather, these mutations are more likely to be associated with nonsense-mediated messenger RNA decay. Chan *et al.* (1994) suggested that complete absence of a keratin is less detrimental than the disrupted filament assembly and aggregate formation caused by dominant-negative missense mutations. The present findings and other reports in the literature agree with this hypothesis in that homozygous missense mutations lead to milder phenotypes with blistering only in the extremities (patients 1, 10, and 11 in Table 1), whereas nonsense mutations are associated with more generalized blistering (patients 2, 3, 4, 5, 7, 8, and 9 in Table 1).

Table 1. *KRT14* mutations in recessive EBS

No.	Phenotype, age	Mutations	References
1.	Blistering affecting the lateral and dorsal aspects of the feet and plants, two patients aged 20 and 12 y	p.E144A/p.E144A	Hovnanian *et al.* (1993)
2.	Severe generalized blistering, 5 y	c.313_314delGC/ c.313_314delGC	Rugg *et al.* (1994)
3.	Generalized blistering, 29 m	p.Y204X/p.Y204X	Chan *et al.* (1994)
4.	Severe generalized blistering, mucous membranes occasionally affected; family with 4 patients aged 74, 67, 47, 34 y	c.1842-2A>C/c.1842-2A>C	Jonkman *et al.* (1996)
5.	Generalized blistering, occasional oral blisters, siblings of 7 and 6 y	p.W305X/p.W305X	Corden *et al.* (1998)
6.	Mild generalized blistering, 18 m	c.92delT/c.92delT p.I31fs86X	Batta *et al.* (2000)
7.	Generalized skin blistering and mild nail involvement and involvement of mucous membranes, 8 y	c.744delC/insAG/ c.744delC/ insAG p.Y248X	Landschuetzer *et al.* (2003)
8.	Widespread blistering and oral mucosal involvement, anaemia and failure to thrive in the first year of life, NA	p.Q396X/p.Q396X	Ciubotaru *et al.* (2003)
9.	Widespread blistering over palms and soles and oral and genital mucosa, NA	p.W305X/p.W305X	Ciubotaru *et al.* (2003)
10.	Blisters over palms and soles, NA	p.Q396X/p.R388H	Ciubotaru *et al.* (2003)
11.	Blisters over hands and feet, 4 y	p.R134C/p.R134C	Indelman *et al.* (2005)
12.	Blisters on hands and feet, later generalized, 2 y	c.427delC/c.427delC p.L143fsX2	This study
13.	Severe blistering of hands and feet, oral erosions, widespread induced blisters, 1 y	c.129dupC/c.129dupC p.S44fsX38	This study

m, months; NA, not available; y, years.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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